WANDA M. KRAJEWSKA and LEOKADIA KŁYSZEJKO-STEFANOWICZ

HIGH MOBILITY GROUP NON-HISTONE CHROMOSOMAL PROTEINS FROM CHICKEN THROMBOCYTES AND ERYTHROCYTES

Department of Cell Structures Biochemistry, Institute of Biochemistry and Biophysics, University of Łódź
Banacha 12/16, 90-237 Łódź, Poland

The high mobility group non-histone chromosomal proteins (HMG) from chicken thrombocytes were compared with those from chicken erythrocytes. In terms of their extractability, electrophoretic behaviour in polyacrylamide gels containing 2.5 M-urea, and amino acid composition, the HMG proteins of these cells were indistinguishable. However, microcomplement fixation assay performed in the presence of antiserum against HMG-1 and HMG-2 revealed that these high molecular weight HMG proteins of chicken thrombocytes and erythrocytes are not cross-reactive. Electrophoretic analysis of HMG-1 and HMG-2 from these two types of cells in polyacrylamide gels containing 6.25 M-urea suggested differences in the HMG-2 patterns.

Avian thrombocytes are considered to be genealogically closely related to erythrocytes. They develop from similar parent cells and possess a number of common morphological features but their cytogenesis remains unknown (Lucas & Jamroz, 1961). It is currently thought that protein components of chromatin may be involved in some aspects of gene regulation and cellular differentiation. Our previous papers on the characterization of chromatin proteins from chicken thrombocytes and erythrocytes seem to confirm the close relationship between these cells although some significant differences were also observed. They concerned mainly the proportions of various protein fractions, their involvement in template activity, the immunospecificity of non-histone chromatin proteins tightly bound to DNA and the chromatin-bound protease activity (Krajewska & Kłyszejko-Stefanowicz, 1980, 1982a,b).

Chromatin isolated from a variety of eukaryotic organisms and tissues contains a class of non-histone proteins known as the high mobility

* This work was supported by the Polish Academy of Sciences within the project 09.7.1.
group¹ chromosomal proteins (Goodwin et al., 1973). The HMG proteins aroused considerable interest because of their possible association with active genes. Since these proteins are present in approximately 10⁷ molecular copies per mammalian cell nucleus, and are also present in inactive cells such as nucleated erythrocytes, it has been suggested that they function not as primary regulatory proteins but as structural components possibly involved in modification of the basic chromatin structure required for replication or transcription (Goodwin & Mathew, 1982). Determination of the specificity of HMG proteins may be helpful in elucidating their role in these cellular processes. The four major HMG proteins, i.e., HMG-1, HMG-2, HMG-14 and HMG-17 were identified in all mammalian and avian species studied (Goodwin et al., 1978; Rabbani et al., 1978a). Comparison of electrophoretic behaviour, amino acid composition, tryptic peptide mapping, isoelectric focusing patterns and immunological relatedness revealed that HMG proteins generally exhibit a striking lack of tissue and species specificity (Rabbani et al., 1978b; Sterner et al., 1978; Romani et al., 1979). Although the HMG-E protein, an equivalent of the HMG-2a or HMG-2B subfraction of HMG-2, was originally suggested to be unique for nucleated red blood cells (Sterner et al., 1978), it was soon shown to be present also in other avian tissues (Mathew et al., 1979; Gordon et al., 1980). Tissue-specific distribution of both total HMG-2 and HMG-2 subfractions was also reported (Mathew et al., 1979; Seyedin & Kistler, 1979; Gordon et al., 1980).

In this paper the HMG proteins from chicken thrombocytes were compared with those from chicken erythrocytes, in terms of their extractability, electrophoretic mobility in acetic acid/urea/polyacrylamide gels and amino acid composition. Additionally, the immunological relatedness of HMG-1 and HMG-2 from these cells was tested by complement fixation.

MATERIALS AND METHODS

Isolation of cells and nuclei. Thrombocytes and erythrocytes were isolated from chicken blood as described previously (Krajewska & Kłyszejko-Stefanowicz, 1980). Nuclei were obtained according to Evans & Lingrel (1969), with the addition of 0.5% Triton X-100 to remove membrane ghosts, and purified by centrifugation through 1.8 m-sucrose for 30 min at 17 000 g (Hardy et al., 1978).

Extraction and purification of HMG proteins. All steps were carried out at 0 - 4°C in the presence of 0.5 mM-PSMF. Purified nuclei were washed three times with 75 mM-NaCl and 25 mM-EDTA, pH 7.5. HMG proteins were extracted with 0.35 M-NaCl, pH 7.0, and the low mobility group

¹ Abbreviations: HMG, high mobility group chromosomal proteins; PMSF, phenylmethysulphonyl fluoride.
non-histone protein was precipitated with 2% trichloroacetic acid (Goodwin et al., 1973). Contaminating histone H1 was removed according to Sterner et al. (1978). Briefly, the crude extract was purified by passage through a column (1×7 cm) of CM-Sephadex C-25 equilibrated with 0.6 M-NaCl, 10 mM-borate buffer, pH 9.0. Under these conditions, the HMG proteins were eluted in the void volume and the contaminants were retained on the column. The HMG proteins were recovered from solution by acetone precipitation (Goodwin et al., 1975).

Polyacrylamide-gel electrophoresis. HMG proteins after dialysis against 8 M-urea, 0.01 M-HCl and 0.5 M-2-mercaptoethanol were separated in 15% polyacrylamide gels, pH 2.7, containing 2.5 or 6.25 M-urea according to Panyim & Chalkley (1969). About 10 μg and 40 μg of protein were applied per gel for analytical and preparative purposes, respectively. Gels were stained with 1% Amido Black 10B in 7% acetic acid/70% ethanol of HMG-1 and HMG-2, which were separated from the total HMG proteins by electrophoresis in acetic acid/polyacrylamide gels containing 2.5 M-urea (Panyim & Chalkley, 1969). Unstained gels were cut according to stained patterns, homogenized and emulsified with complete and subsequently incomplete Freund's adjuvant. Immunization was performed by injecting white New Zealand rabbits in multiple intradermal sites with a total of about 40 - 80 μg protein every week for six weeks. Blood was collected by marginal ear vein bleeding 7 days after the last injection. All sera were heat-inactivated at 56°C for 30 min and kept at -20°C.

Microcomplement fixation technique. The quantitative microcomplement fixation assay was carried out in the presence of 0.14 M-NaCl, 5 mM-MgSO₄, 1.5 mM-CaCl₂, 0.1% bovine serum albumin, 10 mM-Tris/HCl buffer, pH 7.4 (Chytíl, 1977). Washed sheep red blood cells were activated with antishheep red blood cells serum. Guinea pig serum complement was titrated to give 100% lysis of the activated sheep red blood cells after 30 min of incubation at 37°C. The concentration ranges of antigen (total HMG proteins) were incubated for 18 h at 4°C in the presence of titrated complement with 0.1 ml of diluted antiserum. Activated sheep red blood cells were added, and, after 30 min of incubation at 37°C, the extent of red blood cells lysis was measured spectrophotometrically at 413 nm. To determine the optimal tites of antisera a standard amount of antigen was incubated with increasing dilutions of antiserum. All assays were tested for anti-complementarity.

Amino acids analysis. Protein samples (0.5 mg) were dialysed against deionized waer, lyophilized, suspended in 5.5 M-HCl, and hydrolysed at 110°C for 24 h. The hydrolysed samples were evaporated in vacuo and analysed with JEOL JLC-6 (Japan) amino acid analyser. No corrections were made for hydrolytic losses.
Protein estimation. Protein was determined turbidimetrically in the presence of 25% trichloroacetic acid or by the Lowry et al. (1951) method using bovine serum albumin as a standard.

Reagents. These were from the following sources: antisheep red blood cell serum, complete and incomplete Freund’s adjuvant from Bio-Med (Kraków, Poland); guinea pig serum complement from Miles Lab. Inc. (Elkhart, USA); bovine albumin, phenylmethylsulphonyl fluoride and 2-mercaptoethanol from Sigma Chem. Co. (St. Louis, U.S.A.); acrylamide, urea puriss. and Triton X-100 from Koch-Light Lab. Ltd. (Colnbrook, Bucks., England); N,N'-methylenebisacrylamide and ammonium persulphate from Fluka AG (Buchs SG, Switzerland); N,N',N',N'-tetramethylethylenediamine and Amido Black 10B from Serva Feinbiochemica (Heidelberg, F.R.G.); CM-Sephadex C-25 from Pharmacia Fine Chemicals (Uppsala, Sweden). All other chemicals were analytical grade products purchased from POCh (Gliwice, Poland).

RESULTS AND DISCUSSION

Extractability, electrophoretic mobility and amino acid composition are the criteria normally used to identify HMG proteins. The non-histone chromosomal proteins isolated from chicken thrombocytes fulfill all the criteria of HMG proteins. They are extractable with 0.35 m-NaCl and soluble in 2% trichloroacetic acid. They are characterized by high mobility in gel electrophoresis, a high content of charged residues (about 23% basic and 25% acidic amino acids), and a low content of hydrophobic amino acids. HMG proteins of chicken thrombocytes were separated by polyacrylamide-gel electrophoresis in the presence of 2.5 m-urea into four major bands with mobilities identical to those of chicken erythrocyte HMG protein fractions, i.e., HMG-1, HMG-2, HMG-14, and HMG-17 (Plate 1). The amino acid composition of the corresponding HMG proteins from thrombocytes and erythrocytes was found to be remarkably similar (Table 1). Thus, with respect to these properties, HMG proteins of chicken thrombocytes and erythrocytes were indistinguishable.

The convenience of serological techniques for probing the specificity of nuclear proteins (Hnilica & Briggs, 1980) is well established. Since high molecular weight HMG proteins may be expected to exhibit cell specificity, chicken erythrocyte HMG-1 and HMG-2 (separated from total HMG protein in acetic acid/urea polyacrylamide-gel electrophoresis) were used to elicit antibodies. The relationship of thrombocyte and erythrocyte HMG proteins in the presence of antisera was studied by the microcomplement fixation technique. Each of the antisera showed strong complement fixation when tested against the homologous antigen but no positive reaction was observed with HMG proteins from thrombocytes or from calf thymus used as standard
Plate 1. Electrophoretic patterns of HMG proteins from chicken thrombocytes (A) and erythrocytes (B). Electrophoresis was performed in 15% polyacrylamide gels, pH 2.7, containing 2.5 M-urea at 1.5 mA per gel for 4 h (Punyim & Chalkley, 1969). Gels were stained with 1% Amido Black 10B in 7% acetic acid, 20% ethanol. About 10 μg of protein was applied per gel.
Plate 2. Electrophoretic patterns of HMG-I and HMG-2 proteins from chicken erythrocytes (A), chicken thrombocytes (B) and calf thymus (C). Electrophoresis was performed in 15% polyacrylamide gels, pH 2.7, containing 6.25 M-urea at 1 mA per gel for 7 h (Panyim & Chalkley, 1969). Gels were stained with 1% Amido Black 10B in 7% acetic acid, 20% ethanol. About 10 μg of protein was applied per gel.
Table 1

Amino acid composition of the HMG proteins from chicken thrombocytes and erythrocytes

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Thrombocytes</th>
<th>Erythrocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>moles/100 moles recovered</td>
<td></td>
</tr>
<tr>
<td>Lys</td>
<td>18.6</td>
<td>18.6</td>
</tr>
<tr>
<td>His</td>
<td>2.7</td>
<td>2.7</td>
</tr>
<tr>
<td>Arg</td>
<td>3.5</td>
<td>2.7</td>
</tr>
<tr>
<td>Asx</td>
<td>9.1</td>
<td>9.1</td>
</tr>
<tr>
<td>Thr</td>
<td>4.2</td>
<td>3.8</td>
</tr>
<tr>
<td>Ser</td>
<td>6.6</td>
<td>6.3</td>
</tr>
<tr>
<td>Glx</td>
<td>13.7</td>
<td>14.5</td>
</tr>
<tr>
<td>Pro</td>
<td>9.4</td>
<td>8.8</td>
</tr>
<tr>
<td>Gly</td>
<td>8.2</td>
<td>8.7</td>
</tr>
<tr>
<td>Ala</td>
<td>17.7</td>
<td>18.9</td>
</tr>
<tr>
<td>Cys</td>
<td>trace</td>
<td>trace</td>
</tr>
<tr>
<td>Val</td>
<td>2.4</td>
<td>2.3</td>
</tr>
<tr>
<td>Met</td>
<td>trace</td>
<td>trace</td>
</tr>
<tr>
<td>Ile</td>
<td>0.7</td>
<td>0.6</td>
</tr>
<tr>
<td>Leu</td>
<td>2.7</td>
<td>2.5</td>
</tr>
<tr>
<td>Tyr</td>
<td>trace</td>
<td>trace</td>
</tr>
<tr>
<td>Phe</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Asx + Glx</td>
<td>24.8</td>
<td>23.6</td>
</tr>
<tr>
<td>Lys + His + Arg</td>
<td>22.8</td>
<td>24.0</td>
</tr>
</tbody>
</table>

reference (Fig. 1). The specificity of all antisera was also confirmed by testing them against histones. No cross-reaction was detected in the presence of either erythrocyte or thrombocyte histones. These results clearly indicate that HMG-1 and HMG-2 proteins of chicken thrombocytes and erythrocytes are not immunologically cross-reactive.

HMG-1 and HMG-2 proteins from several eukaryotes were compared using serological techniques. Immunological analyses of HMG-1 derived from various sources revealed that these proteins display a remarkable evolutionary conservation in their primary structure. It was established that the sequence differences among HMG-1 from calf thymus, mouse liver and duck erythrocyte are about 3%. On the other hand, HMG-E and HMG-2 appeared to be different proteins with distinguishable antigenic properties. Duck erythrocyte HMG-E and calf thymus HMG-2 differ from HMG-1 in 13% and 8% of the sequences, respectively (Bustin et al., 1978; Romani et al., 1979, 1980). Thus, the observed immunological specificity of high molecular weight HMG proteins from chicken thrombocytes and erythrocytes appears to be due mainly to the different antigenic properties of HMG-2.

Two distinct subfractions of HMG-2 were found to occur in chicken tissues and were designated HMG-E and HMG-2 (Sterner et al., 1978),
HMG-2a and HMG-2b (Mathew et al., 1979) or HMG-2B and HMG-2A (Gordon et al., 1980). They differ in electrophoretic behaviour, isoelectrofocusing patterns, peptide maps, and amino acid composition. Moreover, the relative proportions of the HMG-2 subfractions depend on the tissue. It was found that the more acidic subfraction, equivalent to HMG-E, was predominant in erythrocytes while the more basic one, equivalent to HMG-2, predominated in the thymus (Mathew et al., 1979).

![Graph showing complement fixation of HMG proteins from different tissues.](image)

Fig. 1. Complement fixation of HMG proteins from chicken thrombocytes, chicken erythrocytes and calf thymus in the presence of antiserum (dilution 1:100) against HMG-1 and HMG-2 of chicken erythrocytes.

The differences in patterns of HMG-2 from thrombocytes and erythrocytes were confirmed by polyacrylamide-gel electrophoresis in the presence of 6.25 μM-urea (Plate 2). Electrophoretic mobility of HMG-2 from chicken thrombocytes resembles that of calf thymus rather than of chicken erythrocytes.

Since the presence of distinct HMG-2 subfractions is probably of structural and functional importance, it may be assumed that their cellular function in nucleated thrombocytes and erythrocytes is different.

We wish to express our sincere appreciation to W. Michalak, M.Sc., from the Laboratory of Biological Sciences, University of Łódź for the
amino acid analysis, to Miss M. Radwan for preparation of figures and photographs, and to J. Gierak, M.Sc., for technical assistance and typing of the manuscript.

REFERENCES


Received 5 September, 1984